Longer storage of red blood cells does not affect mortality in transfused liver transplant recipients

Kwon, Ji Hye ; Han, Sangbin ; Cho, Duck ; Spahn, Donat R ; Ko, Justin S ; Kim, Do Yeon ; Jun, Joo Hyun ; Gwak, Mi Sook ; Kim, Gaab Soo

Abstract: BACKGROUND: The characteristics of red blood cell (RBC) products change after 2 weeks of cold storage. It is unclear whether older RBCs affect mortality after liver transplantation. This retrospective cohort study aimed to evaluate the association between the age of transfused RBCs and death after living donor liver transplantation (LDLT). STUDY DESIGN AND METHODS: Of 200 recipients who underwent LDLT, 118 who received RBCs with a mean storage duration of less than 10 days (shorter storage group) were compared with 82 with an RBC mean storage duration of more than 14 days (longer storage group). Key exclusion criteria were transfusion of very fresh RBCs stored for less than 4 days and transfusion of old RBCs in recipients of the shorter storage group. The primary outcome was posttransplant overall death. Survival analysis was performed using the Cox model. RESULTS: Mean RBC storage duration was 7 days in the shorter storage group and 17 days in the longer storage group. Death probability at 1, 2, and 5 years posttransplant was 5.1%, 7.6%, and 13.6% in the shorter storage group, respectively, and 6.1%, 8.5%, and 13.5% in the longer storage group. Death risk was comparable between the two groups in univariable (hazard ratio [HR] 1.00, 95% confidence interval [CI], 0.47-2.16, p = 0.991) and multivariable (HR 1.07, 95% CI, 0.46-2.50, p = 0.882) analyses. Graft failure risk was also comparable (HR 1.04, 95% CI, 0.50-2.18, p = 0.916). Hepatocellular carcinoma recurrence probability at 1, 2, and 5 years was 10.8%, 15.4%, and 23.1%, respectively, in the shorter storage group and 11.4%, 15.9%, and 20.7% in the longer storage group (HR 0.84, 95% CI, 0.37-1.89, p = 0.670). No significant differences were observed regarding graft regeneration/function, vascular/biliary complications, acute kidney injury, surgical site infection, or rejection (p > 0.05). CONCLUSIONS: No evidence was found that transfusion of old RBCs contributes to death after LDLT.

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Ji Hye Kwon,[1] Sangbin Han,[1] Duck Cho, MD,[2] Donat R. Spahn,[3] Justin S. Ko,[1] Do Yeon Kim,[1] Joo Hyun Jun,[4] Mi Sook Gwak,[1] and Gaab Soo Kim[1]

[1]From the Department of Anesthesiology and Pain Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

[2]From the Department of Laboratory Medicine and Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

[3]From the Institute of Anesthesiology, University and University Hospital of Zürich, Zürich, Switzerland

[4]From the Department of Anesthesiology and Pain Medicine, Kangnam Sacred Heart Hospital, Hallym University College of Medicine, Seoul, Korea

Correspondence to (address reprint requests to): Han S, Department of Anesthesiology and Pain Medicine, Samsung Medical Center, Irwon-ro 81, Gangnam-gu, Seoul 06351, Korea. Tel.: +82-2-3410-2470; Fax: +82-2-3410-0361; E-mail: hans5@skku.edu

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Authorship

Ji Hye Kwon designed the study, collected data, analyzed data, and wrote the manuscript
E-mail: jh0828.kwon@samsung.com
Sangbin Han designed the study, collected data, interpreted data, and wrote the manuscript
E-mail: sangbin.han@samsung.com
Duck Cho interpreted data, gave statistical advice, and provided critical revisions
E-mail: duck.cho@samsung.com
Donat R. Spahn interpreted data, gave statistical advice, and provided critical revisions
E-mail: donat.spahn@usz.ch
Justin S. Ko collected data, analyzed data, and wrote the manuscript
E-mail: jsko@skku.edu
Do Yeon Kim collected data, analyzed data, and wrote the manuscript
E-mail: doyeon31.kim@samsung.com
Joo Hyun Jun collected data, analyzed data, and wrote the manuscript
E-mail: ilpleut@naver.com

Mi Sook Gwak contributed to conception, interpreted data, and gave critical revision

E-mail: gwakms@skku.edu

Gaab Soo Kim contributed to conception, acquisition of data, and interpretation of data

E-mail: gskim@skku.edu

ABBREVIATIONS

HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HR = hazard ratio; LDLT = living donor liver; RBC = red blood cell

ABSTRACT

BACKGROUND: The characteristics of red blood cell (RBC) product significantly change after 2 weeks of cold storage. It is unclear whether older RBCs affect mortality after liver transplantation. Thus, in this retrospective cohort study, we aimed to evaluate the association between the age of transfused RBCs and death after living donor liver transplantation (LDLT).

STUDY DESIGN AND METHODS: Of 200 recipients who underwent LDLT, 118 who received RBCs with a mean storage duration of <10 days (shorter-storage group) were compared with 82 with a RBC mean storage duration of >14 days (longer-storage group). Key exclusion criteria were transfusion of very fresh RBCs stored for <4 days and transfusion of old RBCs in recipients of shorter-storage group. The primary outcome was post-transplant overall death. Survival analysis was performed using the Cox model.

RESULTS: Mean RBC storage duration was 7 days in shorter-storage group and 17 days in longer-storage group. Death probability at 1/2/5 years post-transplant was 5.1%/7.6%/13.6% in shorter-storage group, respectively, and 6.1%/8.5%/13.5% in longer-storage group. Death risk was comparable between the two groups in univariable (hazard ratio [HR]=1.00 [0.47–2.16], p=0.991) and multivariable (HR=1.07 [0.46–2.50], p=0.882) analyses. Graft failure risk was also comparable (HR=1.04 [0.50–2.18], p=0.916). Hepatocellular carcinoma recurrence probability at 1/2/5 years was 10.8%/15.4%/23.1%, respectively, in shorter-storage group and 11.4%/15.9%/20.7% in longer-storage group (HR=0.84 [0.37–1.89], p=0.670). There were no significant differences regarding graft regeneration/function, vascular/biliary complications, acute kidney injury, surgical site infection, and rejection (p>0.05).

CONCLUSIONS: We found no evidence that transfusion of old RBCs contributes to death after LDLT.

Despite continuing improvements in surgical and anesthetic techniques, the amount of blood lost during liver transplantation is significant. Consequently, allogeneic red blood cell (RBC) transfusion is sometimes inevitable to maintain a sufficient tissue oxygen supply and save lives. However, RBC transfusion is associated with various immunologic and nonimmunologic effects, and some of them are known to be fatal.[1,2] Thus, various methods, such as blood salvage, leukoreduction, irradiation, and washing, are employed to prevent adverse effects of RBC transfusion in liver transplant recipients.[2,3]

The use or nonuse of RBCs with certain storage durations can also be considered as a method to prevent adverse effects. However, this method should be considered carefully after weighing the expected clinical benefit against the potential for a shortage of RBC products.[4,5] Recently, we found that perioperative transfusion of fresh RBCs stored for <4 days was associated with increased mortality in immunosuppressed liver transplant recipients,[6] being in line with recent evidences demonstrating the risk of blood stored for a short duration.[7] On the other hand, the use of old RBCs also carries potential risks because the structure of the RBCs and the biochemical characteristics of the storage medium change significantly after 2 weeks of cold storage,[8,9] a condition termed the "RBC storage lesion." Although such changes have been found to be associated with vasoconstriction, coagulation disturbance, inflammation, and immunomodulation,[9] the clinical relevance of these changes remains unclear. Recent systematic reviews[10-13] and four large trials have demonstrated no significant impact of old RBCs on mortality in the general hospital population,[14] critically ill population,[15,16] and cardiac surgical population.[17] However, only a limited number of studies have been performed in liver transplant recipients, and the studies have reported conflicting results.[18-20] Therefore, we aimed to reappraise the risk of old RBCs in liver transplant recipients to determine whether there is a clinical benefit from avoiding use of such RBCs.

MATERIALS AND METHODS

Subjects and data collection

We initially screened the records of 480 patients who underwent their first adult-to-adult living donor liver transplantation between January 2006 and May 2014 in our hospital and received at least one RBC unit during the perioperative period, which was defined as the time during surgery and within 2 weeks after transplantation.[6] Of these recipients, we excluded 135 recipients who received at least one RBC unit stored for <4 days.[6] Among the remaining 358 recipients, 111 recipients who received RBCs with a mean storage duration of 10–14 days were further excluded to increase the sensitivity of potential effects of older RBCs and decrease the risk of type II error by increasing the gap in RBC storage duration between study groups. Fourteen recipients who received RBCs with a mean storage duration of <10 days but who also received at least one RBC unit stored for >14 days were further excluded along with 11 recipients who received at least one RBC unit prior to surgery during admission. Nine recipients who were confirmed via pathology to have hepatocellular carcinoma (HCC) macrovascular invasion were further excluded due to the very high risk of death from HCC recurrence.[6,21] Among the remaining 200 recipients, 118 recipients who received RBCs with a mean storage duration of <10 days were included in shorter-storage group and 82 recipients who received RBCs with a mean storage duration of >14 days were included in longer-storage group. No recipients died within 2 weeks of transplantation. Recipients were followed until death or the end of the study in September 2017 for a maximum of 5 years. All data were derived from our institution's electronic medical records and prospectively collected liver transplantation database. The institutional review board (SMC 2017-05-013) approved this retrospective cohort study and waived the requirement for written informed consent.

Operative management

Acceptance criteria for liver donation were ≤65 years, body mass index <35 kg/m^2, macrosteatosis degree ≤30%, and residual liver volume ≥30%. All grafts consisted of segment 5–8 excluding the middle hepatic vein trunk. Graft implantation was performed using the piggyback technique. After portal vein anastomosis, the graft was reperfused by consecutively unclamping the hepatic vein and portal vein. Hepatic artery anastomosis was then performed, followed by biliary anastomosis. Immunosuppression was performed using methylprednisolone, basiliximab, and mycophenolate mofetil in addition to tacrolimus.[21] Tacrolimus treatment was initiated on postoperative day 3, with a trough plasma concentration of 10 ng/mL maintained during the first month and a concentration of 5–8 ng/mL maintained thereafter. Hepatitis B virus (HBV) prophylaxis was performed using hepatitis B immunoglobulin (Green Cross Corp., Yongin, South Korea) until December 2007, and thereafter a combination of entecavir (0.5 mg/day) and hepatitis B immunoglobulin was used.[21] A detailed description of liver donation and transplant criteria, surgical procedures, anesthetic management, immunosuppression, and HBV prophylaxis has been published previously.[21,22]

RBC preparation

RBCs were collected in bags containing citrate-phosphate-dextrose-adenine-1. Prior to transfusion, RBCs underwent post-storage leukoreduction using an RCM1 filter (Haemonetics, Braintree, MA) until March 2012, and thereafter RBCs underwent pre-storage leukoreduction using an RC2VAE filter (Haemonetics). Both filters reduce the number of residual leukocytes per RBC unit to $<2\times10^5$. All RBCs were further gamma-irradiated using our on-site cesium-137 irradiators: an IBL 437 (CIS, Bedford, MA) was used until February 2008, and thereafter, a GammaCell 3000 was used (Best Theratronics, Ottawa, Ontario, Canada). The irradiation duration was modified based on dose distribution mapping

performed by outside experts so that the absorbed-dose at the center of the target was 25 Gy, with a minimum of >15 Gy and maximum of <50 Gy throughout the target (Supplementary Fig. S1). The use of RBCs stored for >24 hours after irradiation was prohibited because of concerns of massive RBC storage lesion (e.g. hyperkalemia) without the consideration of plasma washing.[23] Compatibility was routinely evaluated using ABO/Rh typing, testing recipient serum for clinically important alloantibodies, and serologically cross-matching donor RBCs with recipient serum. Our blood bank delivered RBCs to the operating room using the first-in-first-out principle to consume older RBCs before newer one.[6]

Transfusion policy

The transfusion policy of our transplant team is characterized by restrictive and prophylactic use of blood products.[3,6,22] Each blood component was separately transfused based on its respective indication. The trigger for allogeneic RBC transfusion was hemoglobin 8.0 g/dL. Blood salvage was routinely used for autologous RBC transfusion, with salvaged RBCs being used first when RBC transfusion was indicated. A leukocyte depletion filter was routinely used before autotransfusion for HCC patients.[3] Use of fresh frozen plasma was indicated when the prothrombin time international normalized ratio was >3.0. Platelet transfusion was indicated when the platelet count decreased to $<30\times10^9$/L. Cryoprecipitate was indicated when the fibrinogen level was <80 mg/dL. Korean national policy limits storage of RBCs, platelets, fresh frozen plasma, and cryoprecipitate to 35 days, 5 days, 1 year, and 1 year, respectively. The RBCs, platelets, fresh frozen plasma, and cryoprecipitate were stored at 1–6, 22–26, <-20, and <-20˚C, respectively.

Variables and statistical analyses

The primary outcome was post-transplant death. Survival analyses were performed using the

Cox model, and the results are described using hazard ratio (HR) and 95% confidence interval. The backward selection process was performed for all variables evaluated during the univariable analyses to generate a multivariable model, using $p < 0.05$ for the inclusion of variables and $p > 0.10$ for the removal of variables. The secondary outcomes were graft failure, HCC recurrence, and clinical complications. Graft failure was defined as death or retransplantation. In terms of HCC recurrence, survival analysis was performed using the Fine and Gray model, instead of the Cox model, to account for the competing risk of HCC-unrelated death because HCC-unrelated death significantly affects HCC recurrence probability in liver transplant recipients.[21,24] Post-transplant complications were graded based on the modified Clavien-Dindo classification.[25] The degree of graft regeneration was calculated from the graft volume derived from computed tomography and graft weight directly measured after the graft was procured.[22] Exploratory analysis was performed following inclusion of the 41 recipients who met the inclusion criteria but were excluded because of mean RBC storage duration of 10-14 days. The continuous variables are summarized as median (25th percentile, 75th percentile). The categorical variables are presented as number (%) and analyzed using the chi-square test or Fisher's exact test. All reported p values are two-sided, and a $p < 0.05$ was considered to indicate a statistically significant result. Statistical analyses were performed using SPSS 20.0 (SPSS Inc., Chicago, IL, USA) or R 3.0.2 (R Development Core Team, Vienna, Austria; http://www.R-project.org/).

RESULTS

Among the 1,349 RBC units transfused into the 200 recipients during the 2-week period, 832 units were stored for 4–10 days, and 517 units were stored for >14 days (Fig. 1). Mean RBC

storage duration was 7 (6–8) days in shorter-storage group and 17 (15–19) days in longer-storage group. The number of transfused RBC units did not differ significantly between the two groups (6 [2–8] units in shorter-storage group vs. 6 [4–10] units in longer-storage group, $p = 0.066$). The sum of the storage duration of all transfused RBCs was 40 (19–58) unit-days in shorter-storage group and 110 (62–175) unit-days in longer-storage group ($p < 0.001$). There was a significant difference in recipient ABO blood type ($p < 0.001$): the proportions of A type (27.1% vs. 7.3%) and O type (40.7% vs. 32.9%) were higher in shorter-storage group, while the proportions of B type (25.4% vs. 41.5%) and AB type (6.8% vs. 18.5%) were higher in longer-storage group. The proportion of recipients who underwent transplantation during the post-storage leukoreduction period (the earlier period) was significantly greater in shorter-storage group, indicating that RBC storage duration increased over time in relation to increased blood stock in our hospital blood bank:[5] mean storage duration of RBCs was 9 (7–14) days during the post-storage leukoreduction period and 15 (12–19) days during the pre-storage leukoreduction period. As shown in Table 1, there were no significant differences between the two groups regarding donor/recipient age, donor/recipient gender, macrosteatosis, graft-to-recipient weight ratio, graft ischemia time, donor-recipient ABO blood type compatibility, body mass index, etiology of liver disease, model for end-stage liver disease score, HCC, platelet count, ascites, encephalopathy, or hepatorenal syndrome ($p > 0.05$). Operation time was longer by 31 minutes in longer-storage group (578 vs. 547 minutes, $p = 0.010$). The incidence of intraoperative abrupt massive bleeding was comparable ($p > 0.99$). The amounts of transfused fresh frozen plasma, platelet concentrates, and cryoprecipitate did not differ significantly between the two groups ($p > 0.05$).

After a median follow-up of 60 months, 27 recipients (13.5%) had died. The continuous value of mean RBC storage duration was not associated with death risk (HR=1.01 [0.94–

1.08], p = 0.844). As shown in Fig. 2A, death probability at 1, 2, and 5 years after transplantation was 5.1% (1.0–9.0%), 7.6% (2.7–12.3%), and 13.6% (7.2–19.6%), respectively, in shorter-storage group and 6.1% (0.8–11.1%), 8.5% (2.3–14.4%), and 13.5% (5.7–20.6%), respectively, in longer-storage group. Death risk was comparable between the two groups (HR=1.00 [0.47–2.16], p = 0.991). The presence of HCC beyond the Milan criteria was also associated with death risk (HR=3.90 [1.51–10.06], p = 0.005, Supplementary Table S1). As shown in Table 2, the multivariable analysis confirmed the lack of a significant difference in death risk between shorter-storage and longer-storage groups (HR=1.07 [0.46–2.50], p = 0.882). Only one recipient in each group underwent retransplantation, and accordingly, graft failure risk was also comparable between the two groups (HR=1.04 [0.50–2.18], p = 0.916, Fig. 2B). Within the subgroup of 109 recipients who were confirmed via pathology to have HCC, tumor recurrence probability at 1, 2, and 5 years was 10.8% (3.2–18.47%), 15.4% (6.5–24.2%), and 23.1% (12.7–33.4%), respectively, in shorter-storage group and 11.4% (1.9–20.9%), 15.9% (5.0–6.9%) , and 20.7% (8.5–33.0%), respectively, in longer-storage group, respectively. Recurrence risk did not differ significantly between the two groups (HR=0.84 [0.37–1.89], p = 0.670, Fig. 2C). As shown in Table 3, there were no significant differences in early graft regeneration, early graft function, high-grade complications, hepatic vascular complications, biliary complications, acute kidney injury, surgical site infection, or graft rejection ($p > 0.05$) between the two groups.

After including 41 recipients who were excluded due to borderline mean RBC storage duration (10–14 days), death risk was comparable between shorter-storage group, borderline storage group, and longer-storage group (p = 0.894), indicating selection bias because of the exclusion of recipients with mean RBC storage duration of 10–14 days was not significant (Supplementary Fig. S2A). In addition, 241 recipients were stratified into four groups based on RBC storage quartile strata (4–7 days, n=59; >7 days and ≤10 days, n=66; >10 days and

≤16 days, n=68; and >16 days, n=48). The mean storage duration of each group was 6 (5–7), 9 (8–9), 14 (11–15), and 19 (18–21) days, respectively. Compared to the shortest group, the other groups did not exhibit a significantly different risk in death (p = 0.614). Furthermore, there was no difference in death risk between the longest and shortest storage quartile (HR= 1.5 [0.5–4.2], p = 0.423; Supplementary Fig. S2B).

DISCUSSION

The safety of transfusing old RBCs has long been controversial,[10-12] and conflicting results have been observed in liver transplant recipients.[18-20] In this study, we found no evidence that RBCs stored for a longer time contribute to post-transplant death. Death risk was essentially identical irrespective of the age of RBCs when mean storage duration was treated as a continuous (p = 0.844), dichotomous (p = 0.991), and quartile (p = 0.614) variable. Furthermore, RBC storage duration had no significant effect on graft failure, HCC recurrence, or clinical complications. Our findings are important because practical changes aimed at avoiding use of RBCs stored for longer periods of time is rational only if this practice improves post-transplant clinical courses because such avoidance can increase the potential for a shortage of RBC products. While it is generally accepted that perioperative RBC transfusion negatively affects recipient survival,[26,27] the results from the current study suggest that the use of old RBCs is not a component of that negative impact. Thus, avoiding old RBCs may not decrease post-transplant death following perioperative transfusion. Future validation of our findings is important, along with continuous efforts to distinguish the real components underlying the negative impact of RBC transfusion on liver transplantat recipients.

Because leukocytes and platelets as well as cell-derived free DNA and microvesicles

various cytokines, antibodies, and electrolytes in plasma are simultaneously infused together with RBCs, unwanted effects of RBC transfusion may be observed.[7] The number and property of cells and molecules contained in RBC unit differ by various pre-transplant factors such as blood modification and storage duration.[2,7] With respect to these issues, our study model presented advantages for the robustness of our data. Leukoreduction and irradiation were mandatory.[2] A uniform irradiation dose was applied to our samples, and irradiator quality assurance was performed by external experts.[28] Therefore, in our data, allogeneic leukocyte-related effects were unlikely to raise a significant bias. The timing of irradiation is another issue to be considered because elevated potassium levels in RBC unit stored for long periods following irradiation can lead a deleterious impact on reperfused graft.[29,30] In this regard, the use of RBC units stored for >24 hours after irradiation were prohibited. Furthermore, we excluded RBC units stored for <4 days because these units were determined to significantly increase the risk of post-transplant mortality based on our liver transplant database.[6] Although the underlying mechanisms are now under active investigation, negative impact of fresh RBCs may be associated with higher level of extracelllular free DNA and microvesicles in blood stored for a short duration,[7] in addition to more viable leukocytes.[31,32] Of note, the association between the use of older RBCs and post-transplant mortality significantly changed after including fresh RBCs stored for <4 days (Supplementary Fig. S3), indicating the ease with which results can be distorted by a key pre-transfusion parameter. Finally, an alloantibody test was routine for these units, ruling out the presence of an alloimmunization-related event. This issue is particularly relevant for this population, because liver transplant recipients tend to receive multiple RBC units at multiple times.

Donated RBCs can be stored for as long as 42 days before transfusion, based on the survival of one-quarter of the transfused RBCs during 1 day after transfusion;[2,12] however, one concern is the uncertainty of clinically significant impacts of transfusing older RBCs

stored for different durations within the 42-day allowed time frame. Although conflicting results have been published on this issue, recent systematic reviews concluded that no definitive evidence supports the inferiority of older RBCs.[10-12] Thereafter, the most recent meta-analysis evaluated two new large trials and 10 small previous trials with the shorter-storage group including RBCs stored from 1.6–12.1 days, the longer-storage group including RBCs stored from 9.0–28.3 days, and the gap in RBC storage duration between groups ranging from 7.4–22.5 days, respectively, in mean (or median). This meta-analysis found a consistent lack of significant effects of RBCs with longer storage durations on key outcomes, particularly mortality, concluding that there is no support for avoiding older RBCs.[13] After this meta-analysis, a large, multicenter, randomized trial of patients in the general hospital population demonstrated a lack of significant difference in in-hospital mortality between 6,936 patients with a mean RBC storage duration of 13.0 days and 13,922 patient with a mean RBC storage duration of 23.6 days (odds ratio=1.05 [0.95–1.16], p = 0.34).[14] In the most recent multicenter trial of critically ill patients, there was no significant difference in 90-day mortality between 2,457 patients with a mean RBC storage duration of 11.8 days and 2,462 patients with a mean RBC storage duration of 22.4 days.[16] In liver transplant recipients, however, only three single-center retrospective studies have evaluated the association between older RBCs and post-transplant mortality.[18-20] Although all of these studies used the same cutoff value for old RBCs (2 weeks), the results were different. Unfortunately, neither of the papers specified detailed RBC preparation processes or transfusion practices and only took into account the number of RBC units. Therefore, whether these findings were affected by other transfusion-related factors remains opaque.

Various factors of inventory management may explain the significant difference in RBC storage duration among recipients with different ABO blood types, and these factors may include the age of RBCs at receipt, stock reserve, restock interval, consistent or episodic

transfusion demand, and variance in inventory turnover.[5] That is, higher institutional demand-to-reserve ratio in type O and A may have been involved. Although higher demand may cause a shortage of RBC products, there were no cases in which ABO-compatile RBCs were not available. Results of univariable analysis indicated that ABO blood type was not a significant factor associated with post-transplant death in our study ($p = 0.635$).

This study has several limitations. First, as a retrospective study, we could not exclude the possibility of bias from unobserved (unmeasured or unmeasurable) variables. Nonetheless, rigorous patient selection, uniformly applied blood preparation, and transfusion practices strictly based on institutional protocols along with standardized anesthetic and surgical management helped to minimize the risk for bias. Second, RBCs can be stored for as long as 35 days in South Korea; thus, the impact of RBCs stored for >35 days were not evaluated, even though previous studies reported conflicting results regarding this issue.[33-35] On the other hand, our study had advantages as well. Because the mean RBC storagd duration for the control group (shorter-storage group) was only 7 days, the cells were fresher than those in many previous studies that reported mean RBC storage duration of close to 14 days, a time point when RBC storage lesion is thought to already be significant.

In summary, the risk of post-transplant death, in addition to graft failure, HCC recurrence, and major clinical complications, was not significantly associated with the storage duration of RBCs transfused during the perioperative period. Despite the methodological limitations, our findings suggest that there is no need to change RBC transfusion practices to avoid RBCs stored for longer durations or limit transfusion to fresher RBCs for liver transplant recipients.

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FIGURE LEGENDS

Fig. 1. Histogram of storage duration of transfused red blood cells (RBCs) for 118 recipients in shorter-storage group (gray) and 82 recipients in longer-storage group (black).

Fig. 2. Comparison of post-transplant (A) overall survival, (B) graft survival, and (C) hepatocellular carcinoma (HCC) recurrence.

TABLE 1. Demographic and clinical characteristics of the patients

	Shorter-storage group (n=118)	Longer-storage group (n=82)	p
Graft factors			
Donor age (years)	31 (24-41)	33 (25-47)	0.106
Male donor	113 (67.3)	67 (72.8)	0.353
Blood-unrelated donor	26 (22.0)	23 (28.0)	0.331
Macrosteatsis > 5%	88 (52.4)	44 (47.8)	0.482
Graft-to-recipient weight ratio (%)	1.04 (0.89-1.24)	1.01 (0.89-1.22)	0.725
ABO blood type incompatible graft	16 (9.5)	16 (17.4)	0.065
Graft ischemia time (minutes)	117 (99-137)	118 (98-138)	0.779
Hepatic inflow occlusion			0.417
0	92 (54.8)	46 (50.0)	
1-2	34 (20.2)	16 (17.4)	
≥ 3	42 (25.0)	30 (32.6)	
Recipient factors			
Age (years)	53 (47-57)	53 (47-57)	0.854
Male	129 (76.8)	74 (80.4)	0.496
ABO blood type			<0.001
A	32 (27.1)	6 (7.3)	
O	48 (40.7)	27 (32.9)	
B	30 (25.4)	34 (41.5)	
AB	8 (6.8)	15 (18.3)	
Body mass index (kg/m^2)	24.2 (22.3-26.2)	23.8 (21.4-26.3)	0.494
Hypertension	13 (7.7)	11 (12.0)	0.261
Diabetes	60 (35.7)	29 (31.5)	0.496
Time period*			<0.001
2006-2007	17 (14.4)	5 (6.1)	
2008-2012	92 (78.0)	47 (57.3)	
2012-2014	9 (7.6)	30 (36.6)	
Non-viral etiology	33 (19.6)	22 (23.9)	0.420
Hepatocellular carcinoma			0.720
None	77 (45.8)	44 (47.8)	
Within the Milan criteria	59 (35.1)	28 (30.4)	
Beyond the Milan criteria	32 (19.0)	20 (21.7)	
MELD score	15 (12-23)	14 (10-21)	0.099
Platelet count (×10^9/L)	64 (46-92)	61 (44-104)	0.956
Neutrophil-to-lymphocyte ratio	2.44 (1.67-4.58)	2.14 (1.44-3.64)	0.137
Refractory ascites	40 (23.8)	21 (22.8)	0.858
Hepatic encephalopathy			0.117
None	132 (78.6)	81 (88.0)	
Grade I-III	28 (16.7)	7 (7.6)	
Grade IV	8 (4.8)	4 (4.3)	
Hepatorenal syndrome	9 (7.6)	2 (2.4)	0.205
Transplant factors			
Operative time (minutes)	547 (490-607)	578 (518-639)	0.010
Abrupt massive bleeding events†	6 (9.8)	6 (9.4)	>0.99
Salvaged autologous RBCs (mL)	905 (651-1552)	987 (661-1477)	0.690
Infused autologous RBCs (mL)	816 (456-1550)	828 (299-1312)	0.572
Amount of perioperative transfusion			
RBCs (units)	6 (2-8)	6 (4-10)	0.066
Fresh frozen plasma (units)	4 (2-7)	4 (2-8)	0.919
Platelets (units)‡	8 (0-16)	6 (0-14)	0.515

Cryoprecipitate (units)	6 (0-8)	4 (0-6)	0.500
Tacrolimus concentration >10 ng/mL	75 (44.6)	40 (43.5)	0.857

Data are presented as median (25th percentile, 75th percentile) or frequency (%). RBC, red blood cell. *2006–2007 represents post-storage leukoreduction and hepatitis B virus mono-prophylaxis. 2008–2012 represents post-storage leukoreduction and hepatitis B virus dual-prophylaxis. 2012–2014 represents pre-storage leukoreduction and hepatitis B virus dual-prophylaxis. †Analysis was performed within the subgroup of 125 patients who underwent surgery after May 2009, when data on abrupt massive bleeding began being recorded in the anesthetic chart. ‡One unit of single donor platelet was counted as 6 platelet units.

TABLE 2. Multivariable analysis with post-transplant death as the dependent variable

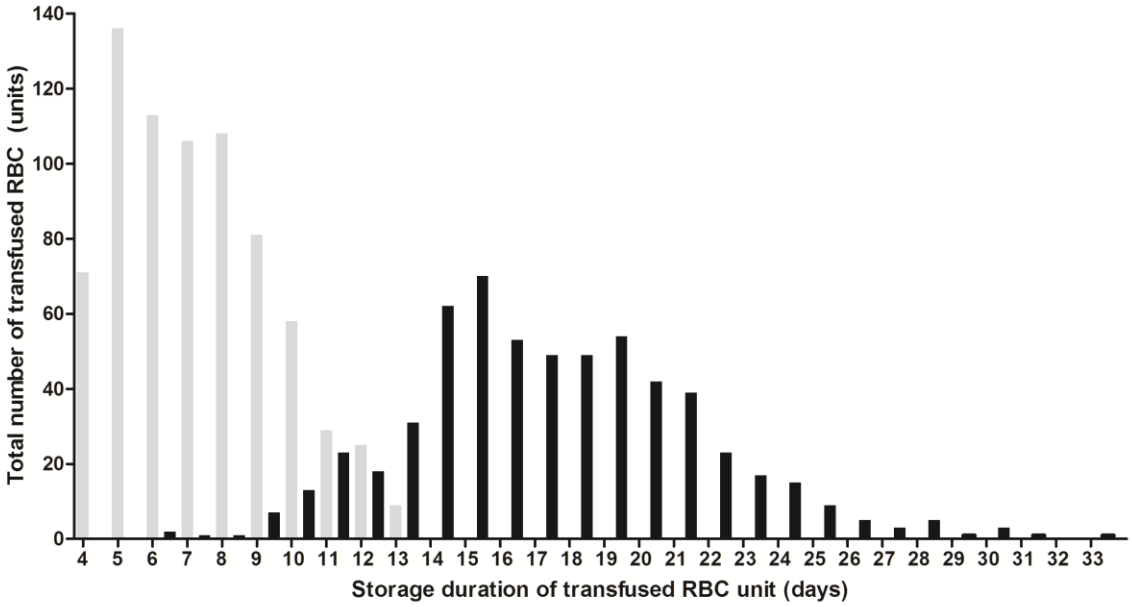
	HR (95% CI)	p
Longer-storage group	1.07 (0.46-2.50)	0.882
Amount of RBC transfusion (units)	1.02 (0.96-1.08)	0.564
Post-storage leukoreduction era (vs. pre-storage)	1.83 (0.56-6.04)	0.319
Male donor	2.72 (0.94-7.90)	0.066
Diabetes	2.07 (0.94-4.56)	0.070
Platelet count ($\times 10^9$/L)	1.01 (1.00-1.02)	0.008
Hepatocellular carcinoma		
Within the Milan criteria (vs. none)	2.25 (0.77-6.62)	0.140
Beyond the Milan criteria (vs. none)	5.06 (1.87-13.74)	0.001
Operative time (minutes)	1.01 (1.00-1.01)	0.012

Backward stepwise selection method was used for generating multivariable model. Infused autologous red blood cell (RBC) amount was not included in the selection process because it is highly similar to salvaged autologous RBC amount. Intraoperative abrupt massive bleeding event was not included in the selection process due to incomplete data (only available in 125 recipients who underwent surgery after May 2009, when the data on abrupt massive bleeding began being recorded in the anesthetic chart). RBC storage duration, perioperative RBC transfusion amount, and leukoreduction method were forced into the multivariable model.

TABLE 3. Postoperative complications, according to the storage duration of red blood cells

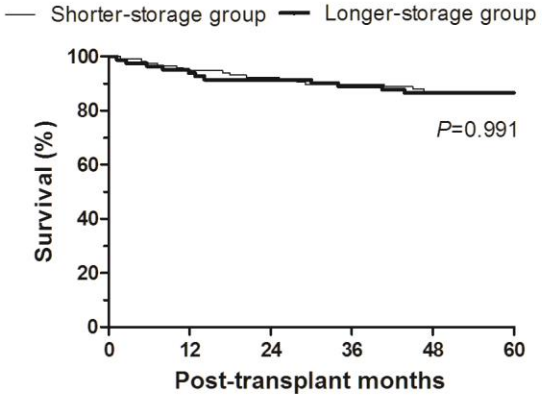
	Shorter-storage group (n=118)	Longer-storage group (n=82)	p
Primary graft non-function	0	0	-
Graft regeneration for 2 weeks (%)	48 (34-67)	52 (39-73)	0.293
AST at postoperative week 2 (IU/l)	41 (27-69)	35 (23-65)	0.196
ALT at postoperative week 2 (IU/l)	157 (87-280)	139 (66-302)	0.592
Prothrombin time at postoperative week 2 (INR)	1.14 (1.06-1.25)	1.16 (1.06-1.27)	0.990
Total bilirubin at postoperative week 2 (mg/dL)	1.4 (1.0-2.1)	1.3 (0.9-2.2)	0.517
Complication III-V	65 (55.1)	50 (61.0)	0.407
Grade IIIb-V	29 (24.6)	27 (32.9)	0.230
Biliary complication (stricture or leakage)	46 (39.0)	36 (43.9)	0.487
Hepatic vascular complication (stricture or thrombosis)	10 (8.5)	9 (11.0)	0.553
Acute kidney injury	48 (40.7)	36 (43.9)	0.650
Grade II to III	14 (11.9)	9 (11.0)	0.846
Surgical site bleeding	6 (5.1)	9 (11.0)	0.120
Surgical site infection	5 (4.2)	4 (4.9)	>0.99
Graft rejection	10 (8.5)	10 (12.2)	0.388

Data are presented as median (25th percentile, 75th percentile) or frequency (%). AST, aspartate transaminase; ALT, alanine transaminase.


Total number of transfused RBC (units)
140
120
100
80
60
40
20
0
4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33
Storage duration of transfused RBC unit (days)

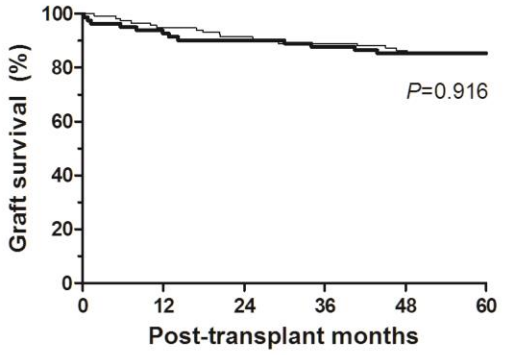
1

A Survival



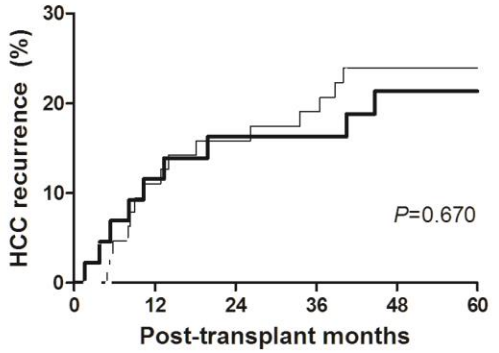
Subjects at risk						
Shorter term	118	113	110	107	101	96
Longer term	82	78	76	72	67	53

B Graft survival



Subjects at risk						
Shorter term	118	113	109	106	100	95
Longer term	82	77	75	73	66	52

C HCC recurrence



Subjects at risk						
Shorter term	65	57	53	51	46	40
Longer term	44	39	36	36	30	21

1

Best Theratronics Dosimetry Report
Absorbed-dose Distribution in a Water-equivalent Medium

GC3000 Serial Number 403

Date of the Irradiation 2016 March 16
Date of the Measurement 2016 May 06

Calculated Timer Setting for 25.0 Gy at the Center: 3 minutes 16 seconds

26.3	23.1	21.7	20.5	20.0	19.7	19.6	19.8	19.9	21.4	23.1	25.7
27.3	23.9	21.5	20.6	20.3	19.6	19.7	20.0	20.3	22.6	23.7	26.8
28.8	24.8	23.6	21.8	21.2	20.9	21.2	21.1	21.6	23.4	24.8	28.5
29.0	26.0	23.7	22.2	22.2	21.5	22.0	21.8	22.2	24.4	25.7	28.8
29.0	25.7	24.0	23.1	22.8	22.3	22.2	22.6	22.2	24.7	26.6	29.4
29.5	26.1	24.7	23.1	23.1	23.1	23.1	23.6	23.4	25.7	27.5	29.9
29.4	26.5	25.2	23.9	24.0	22.8	23.2	23.6	23.6	25.7	27.7	30.1
30.1	26.8	25.7	24.0	23.7	23.7	23.7	24.4	24.7	26.3	27.5	30.9
30.8	27.3	26.3	24.4	24.2	24.4	24.4	24.8	24.8	26.8	28.5	31.5
30.9	28.5	27.0	25.2	24.0	24.8	24.8	25.3	25.5	27.5	28.6	32.0
31.8	27.8	27.7	25.5	25.2	24.8	25.0	25.5	26.0	27.5	28.5	32.2
31.1	27.8	26.5	25.2	25.0	24.4	24.7	25.5	25.5	27.5	28.6	32.0
30.9	28.3	26.5	25.5	25.2	24.8	25.0	25.2	26.0	28.0	28.7	32.6
30.4	27.5	26.5	25.5	24.8	24.2	24.5	24.8	25.0	27.5	29.2	31.6
29.7	27.3	25.8	24.2	24.5	23.6	24.0	24.2	24.5	26.5	28.3	31.5
29.9	27.9	26.0	24.4	24.5	23.7	23.9	24.4	24.7	26.5	27.8	31.5
29.9	26.5	25.5	23.4	23.6	23.2	23.4	23.9	24.0	26.6	28.2	31.7
29.4	26.3	24.8	23.2	22.8	22.5	22.2	23.1	23.1	25.5	27.5	30.9
28.8	25.2	23.2	21.6	22.0	21.4	21.5	22.3	22.9	25.0	27.1	30.6
	24.5	22.3	20.8	21.1	20.5	21.1	20.9	20.9	22.9	25.3	

190 mm

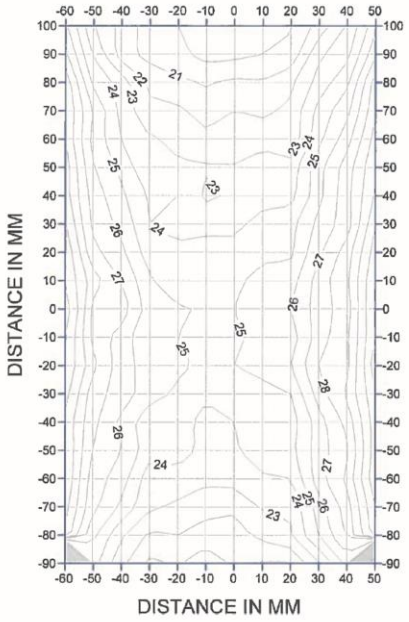
Base
110 mm

Absorbed-dose Measurements in Gy
1 Gy = 100 rad = 100 cGy

9.00-DOS-21 F1 (E)
Page 2 of 3

GC3000 #403

DOSE DISTRIBUTION IN POLYSTYRENE



DOSE VALUES IN Gy
1 Gy = 100 rad
= 100 cGy

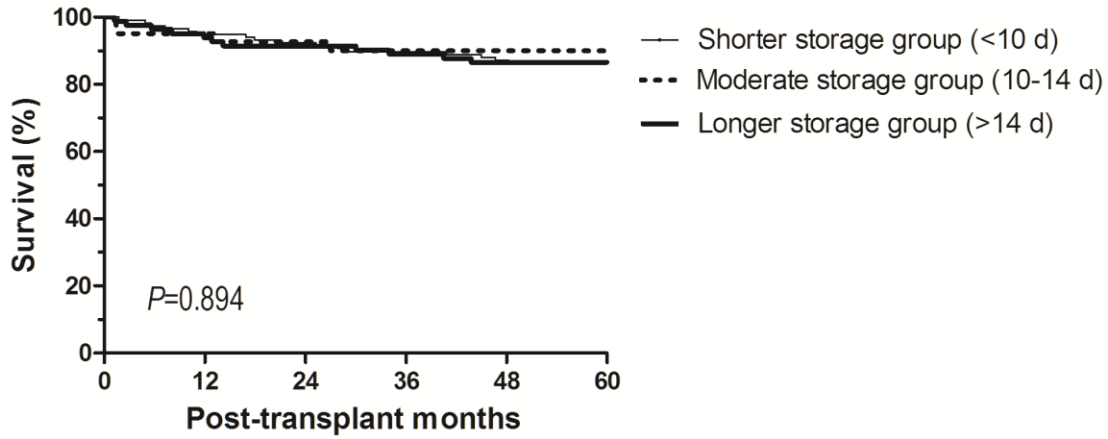
DOSE MAPPING AS OF
2016 March 16

CENTRAL DOSE RATE
9.20 Gy/min.
2008 February 27

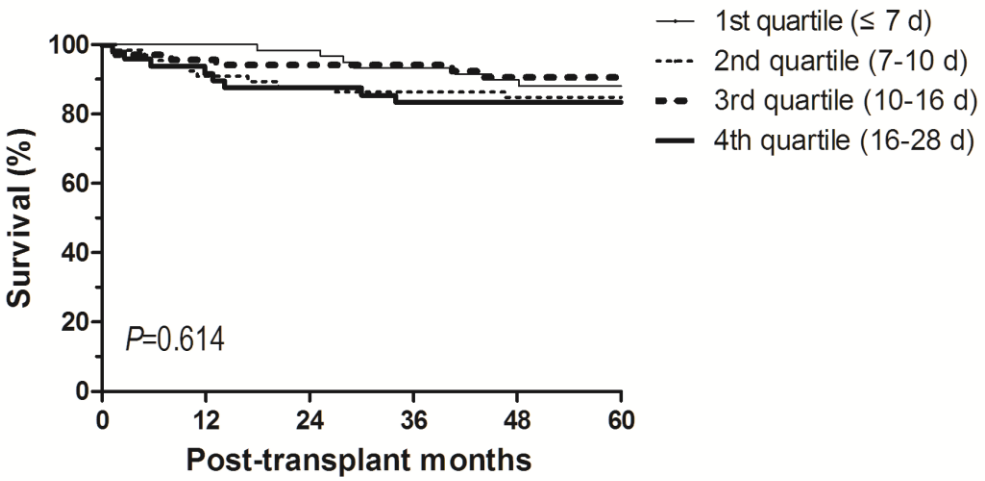
Page 3 of 3
9.00-DOS-21 F1 (E)

1

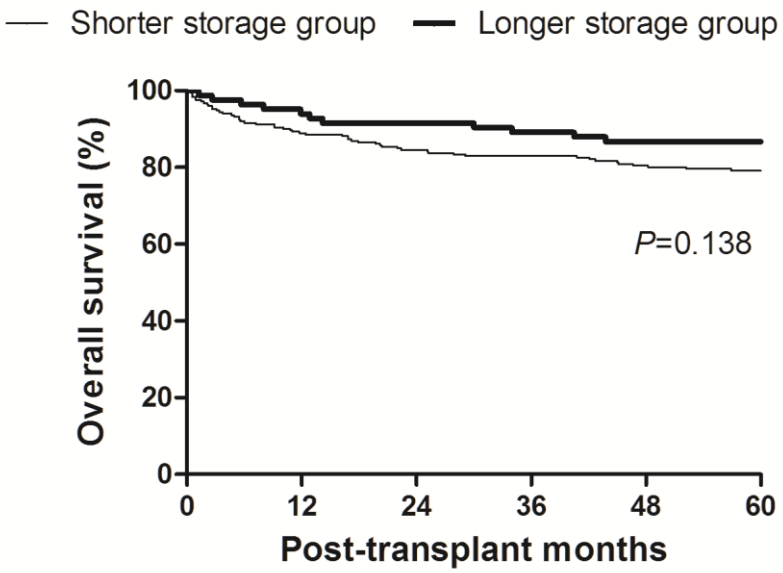
A


Survival (%)
100
80
60
40
20
0
P=0.894
0
12
24
36
48
60
Post-transplant months
Shorter storage group (<10 d)
Moderate storage group (10-14 d)
Longer storage group (>14 d)

B


Survival (%)
100
80
60
40
20
0
P=0.614
0
12
24
36
48
60
Post-transplant months
1st quartile (≤ 7 d)
2nd quartile (7-10 d)
3rd quartile (10-16 d)
4th quartile (16-28 d)

1



Subjects at risk						
Shorter term	252	225	214	206	190	182
Longer term	83	79	77	75	68	54

1